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# The Cell Cycle

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## Introduction

The sustenance of life and growth and the perpetuation of species require organisms to renew and reproduce cells. Conversely, uncontrolled cell division manifests as neoplasia, and can lead to the demise of the organism. At the cellular level, cycles of duplication and equal separation of cellular contents, followed by cell division, accomplish reproduction. These processes are directed by elaborate cell cycle control systems in most species. All forms of life, from single-celled, prokaryotic organisms, such as bacteria, to the most complex, multicellular eukaryotic species are propagated in this manner.

Our understanding of the cell cycle was formerly limited to visual information derived from those structures and events that could be characterized by microscopy. This consisted mainly of the observation of chromatin condensation, the gross description of chromosomal structure, and monitoring of the events that occur on the mitotic apparatus during actual cell division.

Comprehension of the cell cycle has been greatly improved upon in recent years due to advances in molecular biological knowledge and techniques. This has included the elucidation of an intricate system of specific regulatory proteins, which direct and control the cell in its passage through the phases of the cell cycle. Additionally, a system of critical checkpoints in the cell cycle has been described. These checkpoints must be negotiated and passed to allow the cell to successfully continue through the cell cycle, and on to the ultimate goal, which is cell division.

Much of the work leading to the discovery and clarification of these processes was completed in simple, eukaryotic organisms, like budding or fission yeast. Extrapolation of the cell cycle events that occur in yeast to a more complex eukaryotic organism, such as a human, is thought to be valid. This is because the protein-based machinery of the eukaryotic cell cycle control system has been extremely well conserved over many eons of evolution. This is proven in the rescue of mutant yeast cells, which lack one or more protein components of the cell cycle control system. The function of the cell cycle control system can be reestablished in these yeast following transfection with human cell cycle control proteins.

In this review, we discuss the mechanisms and events that comprise the cell cycle. The review will focus on events that occur during the cell cycle in vertebrates. We will analyze the progress and results

of normal cell division, and will briefly examine the derangements in cell cycle control that may allow abnormal and potentially malignant cells to continue to divide.

## Historical Background

The first description of a compound light microscope is credited to Johannes Kepler, circa 1611. Using a compound light microscope in 1655, Joseph Hooke studied cork sections and noted fine fenestrations that he termed "cells." Shortly thereafter, in 1674, the great Dutch amateur scientist Anton van Leeuwenhoek provided the initial microscopic descriptions and drawings of bacteria, yeast, and protozoa. Leeuwenhoek ground his own lenses and mounted them on hand held microscopes of his own design to study these "animalcules." Brown discovered the cell nucleus in 1833, while performing light microscopy on orchids.<sup>1</sup>

A tremendous advance in cell biology occurred in 1838 when Schleiden and Schwann proposed the cell theory. This theory stated that nucleated cells are the basic compositional unit of plant and animal tissues. Several years later, in 1857 Kolliker observed mitochondria in skeletal muscle, and thus became the first investigator to describe an organelle. In 1879, Fleming provided detailed descriptions of chromosomal activity during mitosis.<sup>2</sup>

A century and a score of years have passed since Fleming's discovery. In that time, the fields of cell and molecular biology have grown exponentially. Myriads of advances in light and electron microscopy have occurred. These advances have been accompanied by numerous landmark achievements in tissue culturing, ultracentrifugation, X-ray crystallography, protein electrophoresis, monoclonal antibody technologies, and enzymology. Each of these specialized disciplines has been instrumental in the elucidation of the complex events of the cell cycle.

One of the initial events in the discovery of the cell cycle occurred in 1951 when Howard and Pelc used <sup>32</sup>P to label the roots of the *Vicia faba* seedlings. They noted the period between mitosis and DNA synthesis was relatively prolonged, compared to the period between DNA synthesis and mitosis.<sup>3</sup> This work led to the concept of the four phases of the cell cycle. In 1962 Cohen purified the first known growth factor, epidermal growth factor (EGF).<sup>4</sup> Growth factors subsequently proved to be the substances responsible for providing the impetus for mammalian cells to enter and complete the cell cycle. These were the founding descriptive events of the cell cycle and these discoveries formed the foundation for all future cell cycle research. Although much progress has been made, the cell cycle is far from being completely described.

## An Overview of the Cell Cycle

In its entirety, the cell cycle appears as a continuum of events that lead up to and culminate in cell division. However, the rates at which

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the steps comprising the cell cycle are completed are not uniform. The time it takes for a cell to complete an entire cell cycle and divide varies considerably from species to species, and even from cell type to cell type within the same organism. Variation in the length of the cell cycle also depends on the stage of development of the organism (e.g. fetus vs. adult).

If we dissect the cell cycle into distinct steps, we can see that each step, or **phase**, focuses on specific goals to help prepare the cell for reproduction.<sup>5</sup> Again, time is not divided evenly between the steps of the cell cycle. Thus, some phases are completed much more rapidly than others. This will be discussed in detail below. Some other influences on the speed of the cell cycle include the age of the cell, the age of the organism as a whole, and various signals from the extracellular environment.

The cell cycle of a eukaryotic organism can be divided into four phases. These phases include **mitosis (M phase)**, the **G1 phase**, the phase of **DNA synthesis (S phase)** and the **G2 phase**. During mitosis, the cell divides into two equal daughter cells. The events comprising mitosis may be subdivided further into six separate stages, including **prophase**, **prometaphase**, **metaphase**, **anaphase**, **telophase**, and **cytokinesis**.<sup>6</sup>

The G1, S, and G2 phases of the cell cycle can be grouped into a single period termed **interphase**. During interphase the cell is continuously growing, as long as it continues to progress through the cell cycle toward mitosis. The G1 phase is defined by the **gap** in time between the completion of cell division, and the beginning of DNA synthesis. A cell is in S phase while it is **synthesizing new DNA** in preparation for cell division. The G2 phase is defined by the **gap** in time between the completion of DNA replication and mitosis.

Although the G1 and G2 phases were originally named for the apparent gaps in time between the significant and microscopically observable cellular events that revolve around chromatin condensation and mitosis during S phase and M phase, it is now known that these phases are characterized by intense activity. During the G1 and G2 phases in actively cycling cells, the cell is continuously monitoring its **surrounding environment** and its own **growth**, **RNA synthesis**, and **protein synthesis**. The cell also analyzes the process of **DNA replication** for completion and precision. As the cell nears completion of the G1 and G2 phases, it must pass important **checkpoints**.<sup>7</sup> The cell pauses at these checkpoints to make a definitive analysis of the factors mentioned above. If all is satisfactory, the cell cycle control system propels the cell forward in the cycle, and it is allowed to progress to the S phase or M phase, respectively.

In contrast, cells may enter a special “**resting**” phase called **G0**. Cells in G0 are not actively passing through the cell cycle. In simple eukaryotes, like yeast, this is often due primarily to lack of nutrients, or a particularly hostile extracellular environment. Under optimal conditions, these primitive cells will pass continuously through the cell cycle and divide. In advanced, multicellular organisms, lack of nutrients at the cellular level is not a common problem. Yet, at least in mature adults, most differentiated cell types rarely divide. These cells require specific stimuli to prompt them to re-enter the cell cycle and divide again. Therefore, the rate-limiting factor for cell division in these instances is the presence of a stimulus, such as one or more **growth factors** in sufficient concentrations, which are **specific to the cell type**.

Cells decide to enter G0, or to progress through completion of the cell cycle, near the checkpoint which heralds the end of the G1 phase. Thus, it is the **duration of the G1 phase** that accounts for the great majority of the **variability of time** which different cell types require **to complete the cell cycle**. In slowly dividing cells, the G1 phase may last months to years. Cells that no longer divide remain arrested in the G1 phase.

Before an adequate understanding of the events that occur during each of the phases of the cell cycle is possible, we must acquire insight into the complex control system that regulates a cell's passage through the cell cycle. The following sections will elaborate on the cell cycle control system. We will concentrate on the events that occur in the period between mitoses known as interphase. Mitosis will be discussed in its own section to follow.

## **Interphase**

### **The Cell Cycle Control System**

The cell cycle control system consists of distinct sets of proteins and protein kinases, which are enzymes that phosphorylate proteins to active or inactive forms. Both function specifically to guide the progression of the cell through the cell cycle with control as their explicit function. They do not actively participate in the synthesis of cellular protein or genetic material. These proteins and protein kinases are the primary regulators that determine whether or not a cell passes through the major checkpoints which are found at the end of the G1 and G2 phases, and during metaphase. Passage through these checkpoints is required for the cell to continue through the phases of DNA replication (S phase), and cell division (M phase).

The components of the cell cycle control system are influenced by the **intracellular milieu** and various **environmental factors** through **feedback signals**. These feedback signals relay information on the status of protein or DNA synthesis, cell size, and the environmental conditions with respect to cell division. Abnormal or delayed protein or DNA synthesis, insufficient cell growth, or unfavorable environmental conditions will delay or halt the progress of the cell cycle control system. The control system in turn delays or halts the progress of the cell through the cell cycle. In this fashion, cellular and environmental factors provide important feedback information, and directly influence the control of the cell cycle at the G1 and G2 checkpoints.<sup>8</sup> The checkpoint that occurs at metaphase is concerned primarily with the alignment of chromosomes on the mitotic spindle. Overall, the feedback and checkpoint systems help to prevent lethal or mutagenic cellular catastrophes like entry into mitosis before completion of DNA synthesis, or initiation of anaphase before the chromosomes are aligned properly on the mitotic spindle.

For example, the protein product of the **p53** gene is instrumental in halting the cell cycle in cells with abnormal or damaged DNA before they enter S phase.<sup>9</sup> In cells with damaged DNA, p53 **accumulates during G1**, and brakes the cell cycle in the G1 phase. Many human malignancies have been associated with mutations in the p53 gene.<sup>10</sup> **Loss of function** of the p53 gene results in loss of feedback control, and allows cells with abnormal DNA to pass the G1 checkpoint, and progress through the cell cycle.

As mentioned above, two major classes of proteins comprise the machinery of the cell cycle control system. The **cyclin-dependent protein kinases (Cdks)** influence the progression of the cell cycle by phosphorylating serine and threonine moieties on select proteins

that control the synthesis of cellular elements necessary for reproduction.<sup>11</sup> In yeast cells, a single type of Cdk protein is responsible for cell cycle control. In mammalian systems, there appear to be separate Cdk proteins that are specific for the G1 and G2 checkpoints.<sup>12</sup>

The Cdks are complemented by a group of proteins called **cyclins**.<sup>13,14</sup> Cyclins are synthesized and degraded as the cell goes through the cell cycle. After they are synthesized, the **cyclins bind to Cdk protein molecules** promoting the phosphorylation of target proteins by Cdk. Subsequently, the cyclins are degraded. The Cdk proteins are released, and Cdk phosphorylation of its target proteins is decreased or stopped. Distinct classes of cyclins are synthesized during the G1 and G2 phases.<sup>15</sup> Each subset of cyclins is synthesized and degraded once during the cell cycle, and is designed to work during a specific phase in the cell cycle.<sup>16</sup>

The first class of cyclins to be synthesized following mitosis is the **G1 cyclins**. As might be expected, the G1 cyclins bind Cdk protein molecules during the G1 phase.<sup>17</sup> Formation of this **G1 cyclin-Cdk complex** is necessary for the cell to pass the G1 checkpoint. Once it has passed the G1 checkpoint, the cell enters S phase, and begins a new round of DNA replication.

During the G2 phase, a second class of cyclins, known as **mitotic cyclins**, accumulate and bind Cdk protein molecules.<sup>18,19</sup> This event is extremely important and results in the formation of what is called the **M phase promoting factor, (MPF)**. This factor itself is rather inert, however, other enzymes act to phosphorylate and dephosphorylate the complex, resulting in its activation. Just prior to entering mitosis, cells experience an exponential increase in the concentration of activated MPF. This is thought to be due to a **positive feedback** mechanism in which activated MPF stimulates increased activity of the enzyme or enzymes activating it. In short, activated MPF concentrations build rapidly, and at an accelerating pace until some "critical mass" is reached. The result is that the cell is catapulted into the series of events known as mitosis. Somewhere at the junction between metaphase and anaphase, activated MPF is rapidly inactivated. This is triggered by the degradation of mitotic cyclins. The cell then completes and exits mitosis, and returns to the G1 phase.

### Control of the Vertebrate Cell Cycle

Progression through the cell cycle and reproduction are constitutive features of single-celled organisms, and are influenced mainly by the availability of nutrients. Unless nutrients are unavailable, these primitive organisms are continuously advancing through the cell cycle, dividing, and cycling again. This method assures survival of these species, which are in constant competition against other microorganisms for nutrient resources, and favorable environmental conditions.

For more advanced, multicellular organisms, such as vertebrates, complex signals are required to induce cells to enter the cell cycle and divide. Particularly in adults, most differentiated cells are committed to performing their specialized functions, and are not actively progressing through the cell cycle. There are many different types of signals that may induce a vertebrate cell to divide. Among the most important are the proteins called **growth factors**. Growth factors will be discussed further below, and in detail in another article in this Molecular Biology Review Series. For now, suffice it

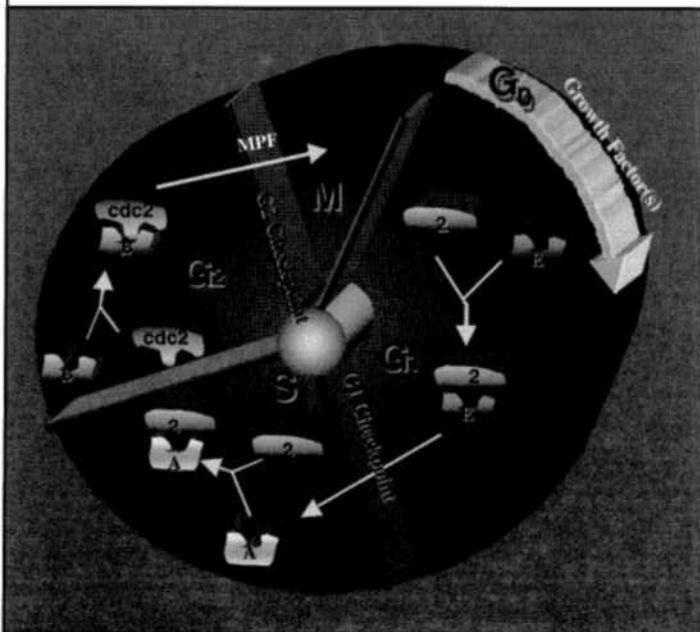
to say that growth factors can generate signals that are transduced into the cell, and can override a cell's inherent tendency to remain in the resting (G0) state. Susceptible cells are induced to enter the cell cycle again and divide.

The structure of the cell cycle control system in vertebrates is fundamentally the same as that in their eukaryotic relatives, the yeasts. However, in vertebrates, the proteins are more numerous, and their functions more phase-specific. Yeasts go through the cell cycle under the control of a single type of Cdk molecule, and two known cyclins. In the vertebrate cell cycle control system, at least **eight cyclins (A, B, C, D, E, F, G, and H)**, and **eight cyclin-dependent kinases (cdc2 and CDK 2,3,4,5,6,7 and 8)** have been identified.<sup>20</sup>

The activation of cyclin-dependent kinases depends on their binding with one or more types of cyclin protein molecules. In the vertebrate cell cycle, passage of the **G1 checkpoint** requires the binding of **CDK2 protein** with **cyclin E**. Activation of **DNA synthesis** requires **CDK2 protein** binding with **cyclin A**. Entry into **M phase** requires **cdc2** to be complexed with **cyclin B**. The **rate-limiting step** appears to be the **synthesis of the appropriate cyclin**.<sup>21</sup> CDK protein molecule concentrations remain relatively stable in cycling cells, while the concentrations of the various cyclins increase in proximity to the cell cycle phase during which they function, and decline thereafter. (Figure 1.)

Additional control of the cell cycle is achieved via the **CDK inhibitors (CDI)**. Two families have been described. The first is the universal CDI that inhibits all of the G1 kinases (CDK2,3,4,6) and is composed of **p21, p27, and p57**. The other family known as the **INK4** family is restricted to inhibition of CDK 4 and 6 and is made up of **p15, p16, p18, and p19**.<sup>22</sup>

Figure 1.— Simplified Cell Cycle: Phases, Cyclins, Cdks, Checkpoints



## **The Checkpoints**

The major checkpoints in the cell cycle occur at the transition from G1 to S phase, and at the transition from G2 to M phase.<sup>23</sup> (Figure 1.) There is also a checkpoint that is found at the transition from metaphase to anaphase, and allows the cell to complete mitosis.

A characteristic common to both yeast and mammalian cells is that they must grow before they can divide. The cytoplasmic volume, and the requisite complement of proteins, and other necessary molecules must be doubled, along with the cell's genetic information, before the cell can divide into two equal daughter cells. This process of cell growth is an extremely important and time-consuming component of the cell cycle. In fact, the time taken to complete adequate cell growth is greater than the total time for both DNA synthesis during S phase and mitosis. The **size checkpoints** at the ends of the G1 and G2 phases are designed to allow the cell to inspect for adequate growth and synthesis of cellular materials. If the cell has grown sufficiently, the control system allows it to proceed through the G1 checkpoint to S phase, or subsequently, through the G2 checkpoint to mitosis. If the cell has not attained adequate size, or if environmental conditions mitigate against cell division, the cell cycle control system halts the cell's progress toward division at the G1 or G2 checkpoint.

In the majority of eukaryotic organisms, the **most important checkpoint** is the **G1 checkpoint**. In yeast cells, this is known as the **Start checkpoint**.<sup>24</sup> In budding yeast cells, as well as most multicellular eukaryotes, passage of the Start checkpoint commits the cell to completion of the cell cycle. This is true even if subsequent environmental or growth circumstances are unfavorable for cell growth and division. Thus, passing Start is equivalent to going beyond the "point of no return." As described above, combinations of appropriate cyclins and Cdks in adequate concentrations are necessary to enable passage of a checkpoint.

The binding of specific cyclins to specific Cdks at each of the major checkpoints results in the formation of important protein kinases. At the **Start (G1) checkpoint**, the protein kinase is called **Start kinase**.<sup>25</sup> At the **G2 checkpoint**, the protein kinase product is the **MPF**. Each of these kinases phosphorylates target proteins that are necessary to take the cell through S phase and M phase, respectively. MPF must be activated by other protein kinases before it can influence the cell to enter mitosis.<sup>26</sup>

## **The Influence of Growth Factors**

Early attempts at mammalian cell cultures failed unless the cells were supplied with serum. This is because mammalian cells will grow and divide only if supplied with **growth factors**, which are present in the serum. This observation eventually led to the isolation of about fifty growth factors. Most growth factors are protein molecules. Other molecules, such as steroid hormones, may function as growth factors as well.<sup>27</sup>

One of the first growth factors to be isolated was **platelet-derived growth factor (PDGF)**.<sup>28</sup> PDGF is produced by platelets and stored in specific granules which are released in response to specific stimuli. One of the primary stimuli for platelet granule release is participation in the hemostatic process. Active hemostasis is typically found in areas of tissue damage or trauma, and release of PDGF is thought to play an important role in the initiation of the subsequent healing process. Among its many functions in this regard are the

induction of fibroblast migration and proliferation.<sup>29</sup>

In regard to the cell cycle, some growth factors influence the rate of protein synthesis, and cell growth. Other growth factors function to aid in ushering cells through the G1 checkpoint without specifically regulating protein synthesis and cell growth.

Growth factors normally circulate in very small concentrations, on the order of nanomoles or picomoles. This makes their isolation very difficult. However, most growth factors are thought to function in a paracrine fashion, being produced and acting locally where needed.

In order to mediate their effects, growth factors must bind to growth factor receptors on the surface of cells. The type of growth factor receptors that a cell possesses will dictate the growth factors to which it is sensitive. Some growth factors like PDGF and **epidermal growth factor (EGF)** are broad-spectrum growth factors.<sup>30</sup> This means that many cell types, including smooth muscle, neuroglia, and fibroblasts express receptors for PDGF. Many types of epithelial and even nonepithelial cells have receptors for EGF. In contrast, some growth factors are very specific. For example, **erythropoietin** receptors are found only on red blood cell precursors. Finally, the growth and proliferation of most cells depends on the influence of multiple growth factors. Therefore, cells will usually express receptors for many different types of growth factors.<sup>31</sup>

When growth factors bind to their transmembrane receptors, the cytosolic portion of the receptor activates various protein kinases that induces a signal transduction process which eventually sends messages to the nucleus. Within the nucleus, alterations in gene expression occur. Specifically, expression of two classes of genes is induced.

The first class is the **early response genes**, whose induction occurs within 15 minutes of growth factor-receptor binding. Synthesis of new proteins is not required to induce expression of this class of genes. However, when activated, these genes direct the synthesis of several proteins. Activation of the early response gene *myc*, results in production of the protein **Myc**.<sup>32</sup> This protein is crucial, as its synthesis is important in transcription of the **delayed response genes**.

Expression of the delayed response genes occurs more than one hour after growth factor-receptor binding, and does require previous synthesis of new regulatory proteins, like Myc. In turn, the protein products that results from delayed response gene expression include the cell cycle control proteins, the cyclins and Cdks.

## **Growth Factors, Serum Deprivation, and the Cell Cycle Control System**

If cells in culture media are deprived of serum at the right point in time, their ability to progress through the cell cycle is greatly impaired. Even short periods of serum deprivation, like one hour, will result in long delays in completion of the cell cycle.

This effect depends on the time when serum deprivation occurs. If fibroblasts are deprived of serum less than 3.5 hours after completing mitosis, they will take an extra eight hours to complete the next cell cycle. If serum deprivation occurs beyond 3.5 hours, the cells will continue through the cell cycle unabated. These observations places the G1 checkpoint somewhere around the 3.5 hour mark after mitosis in these actively cycling cells.

Serum deprivation results in depressed rates of protein synthesis and cell growth. Among the proteins whose synthesis is impacted are the proteins comprising the cell cycle control system. When serum deprivation occurs, cyclins and Cdks are rapidly broken down. After reintroduction of serum, synthesis of new cyclins and Cdks in concentrations sufficient to drive the cell through G1 requires a significant amount of time. This is because the pathway described in the section above must be re-entered. Growth factors must bind their receptors and start the kinase pathway that results in the growth factor message reaching the nucleus. Early response genes can then be transcribed and direct the synthesis of proteins such as Myc. Next, the delayed response genes are transcribed and begin to orchestrate the synthesis of the cell cycle control proteins. These account for the increased time required by these cells to complete the cell cycle.

### **Protooncogenes, Oncogenes and Tumor Suppressor Genes**

**Protooncogenes** promote the growth of the cell, the synthesis of cell cycle control proteins, and guide the cell past the G1 checkpoint. An example of a protooncogene is *myc*. Properly functioning protooncogenes direct the growth and proliferation of cells when appropriate signals, like growth factors are present. Mutated protooncogenes are called **oncogenes**. Oncogenes can induce cellular proliferation beyond the normal constraints of cell control, a primary feature of neoplasia.<sup>33</sup>

**Tumor suppressor genes** are the antithesis of protooncogenes. They inhibit cell proliferation, mainly by braking the cell cycle control system. Mutations in tumor suppressor genes can also result in neoplasia.<sup>34</sup>

One of the earliest tumor suppressor genes to be identified was the **retinoblastoma gene**, which is responsible for directing the synthesis of the **retinoblastoma protein (Rb)**. When it is dephosphorylated, the Rb protein binds regulatory proteins that promote cell proliferation and inhibits them. Phosphorylation of Rb releases these regulatory proteins, and allows them to carry out their functions.

In cells that are in G0, Rb is mainly dephosphorylated. In this state, Rb impairs the transcription of protooncogenes like *myc*, *jun*, and *fos*.<sup>35</sup> Therefore, it brakes the cell cycle and holds cell proliferation in check. Dephosphorylated Rb may also function as part of the braking mechanism that makes up the G1, or Start, checkpoint.

The introduction of growth factors causes the stimulation of phosphorylase and kinase proteins, which result in the phosphorylation of the Rb protein on serine and threonine residues. The cascade of early and delayed response genes and their protein products is activated, and the machinery of the cell cycle control system, including cyclins and Cdks, is assembled. Cells are then driven past the G1 checkpoint, to DNA synthesis, and mitosis.

Loss of both copies of the retinoblastoma gene can therefore lead to uncontrolled cell cycling and reproduction. Clinically, this is seen as abnormal cell proliferation in the immature retina.

## **Mitosis**

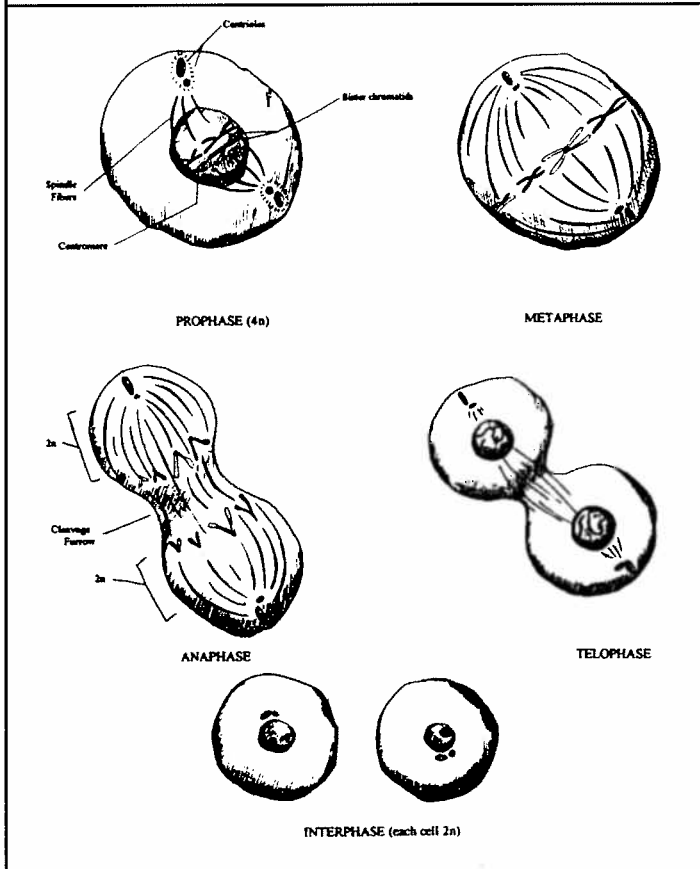
### **An Overview of Mitosis**

The events of interphase are designed to prepare the cell for division. In actively cycling cells, mitosis, or **M phase** is the culmination of the cell cycle. The discussion that follows describes the events of

mitosis that occur in eukaryotic cells.

During mitosis, specialized cytoskeletal rearrangements occur which result in the successive formation, utilization, and dissolution of the **mitotic apparatus**. The major elements of the mitotic apparatus are the mitotic spindle and the contractile ring. The net result is that the genetic material, cytoplasm, and its contents, which have been duplicated during interphase, are divided between two daughter cells. Specifically, mitosis is represented by segregation of pairs of chromosomes, and the production of two separate nuclei. The actual division of the parent cell into two separate daughter cells is called **cytokinesis**. (Figure 2.)

Figure 2.— Phases of Mitosis



### **Key Events in Mitosis**

**Chromosome condensation** heralds the advent of mitosis. This event is associated with the phosphorylation of histone H1 proteins by the enzymatic action of the MPF. This action of the MPF on histone proteins is thought to help direct chromosome condensation.

Next, cytoskeletal microtubules undergo arrangement into a **mitotic spindle**. This spindle helps orient the pairs of chromosomes along an axis that will facilitate distribution of one copy of each chromosome to each daughter cell. As mitosis progresses, the mitotic spindle directs the movements of the daughter chromosomes to opposite spindle poles.<sup>36</sup>

The poles of the mitotic spindle are composed of **centrosomes** and their associated pairs of **centrioles**. The centrosome serves as an

anchor for the microtubular elements of the mitotic spindle. During interphase, the cell synthesizes a centrosome for each daughter cell, but these centrosome copies remain in the proximity of one another in the nucleus, until the onset of mitosis. During mitosis, the nuclear envelope dissolves, and the centrosomes part to migrate in opposite directions. Next, the centrioles begin the task of organizing the microtubules of the mitotic spindle. These microtubules emanate from the centriole pairs in a radial arrangement that is called an aster. The chromosomes migrate along the spindle, and the nuclear envelope reforms. Each daughter cell receives a centrosome and a pair of centrioles with which to begin the next interphase.

Cytoskeletal actin and myosin-2 filaments arrange themselves to form the **contractile ring**. This ring is constructed circumferentially around the cell, just inside the plasma membrane, in a plane that is oriented perpendicular to the mitotic spindle. During mitosis, the contractile ring slowly draws the plasma membrane in the middle of the cell together to form an hourglass-like shape. At the narrowest point, the plasma membrane is pinched together and two daughter cells are created. This is cytokinesis, which is designed to lag slightly behind mitosis.

### **The Phases of Mitosis**

For the purposes of this discussion, we will divide the events into six phases, including: **prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis.**

The most significant event in prophase is the gradual condensation of the dispersed, nebulous nuclear chromatin characteristic of interphase into well-defined, compacted chromosomes. During the preceding S phase, the chromosomes would have undergone replication, and appear during prophase as pairs of sister **chromatids**. Located in each of these chromatids is a DNA sequence known as the **centromere**, which is essential for precise segregation of the sister chromatids. Nucleoli disappear during prophase. The mitotic spindle, composed of cytoskeletal microtubules and their associated proteins, begins to form outside the nucleus near the conclusion of prophase.

Dissolution of the **nuclear envelope** sharply demarcates the transition from prophase to **prometaphase**. After the nuclear envelope dissolves, the developing mitotic spindle comes to occupy its position. Remnants of the nuclear envelope may be seen as membrane vesicles in the vicinity of the mitotic spindle. The centromeres express proteins called **kinetochores**, which attach them to microtubules on the mitotic spindle. The microtubules which have chromatids attached are therefore called **kinetochore microtubules**. The kinetochore microtubules exert tension on the pairs of sister chromatids, which helps them to flagellate, and prepare for segregation. Sister chromatids from each pair attach to kinetochore microtubules originating from the opposite mitotic poles. This ensures that each daughter cell will receive a copy of the chromosome. Those microtubules which are part of the mitotic spindle but do not have chromatids attached are termed **polar microtubules**, and those external to the spindle are known as **astral microtubules**. As prometaphase ends, the kinetochore microtubules are completing the alignments of pairs of chromatids on the mitotic spindle.

In **metaphase**, pairs of sister chromatids are aligned on the mitotic spindle at a point midway between the spindle poles called the **metaphase plate**. The chromatids are attached to the metaphase

plate by their kinetochores, and are kept in alignment by the kinetochore microtubules. Control mechanisms ensure proper alignment of the chromatids on the metaphase plate before the cell is allowed to exit metaphase.

Signaled by **MPP inactivation** and **cyclin degradation**, the cell suddenly enters anaphase. The two kinetochores of each chromosome begin migration toward opposite spindle poles, each pulling a sister chromatid along with it. At this point, each chromatid may be properly termed a **daughter chromosome**.

Migration of the daughter chromosomes to the poles of the mitotic spindle heralds the arrival of **telophase**. Other telophase events include dissolution of kinetochore microtubules, elongation of polar microtubules, and **reformation** of a **nuclear envelope** around each set of daughter chromosomes. Finally, the chromatin diffuses and the nucleoli reappear. Mitosis is then concluded.

Division of the cytoplasm, called **cleavage**, is first noticeable during anaphase. This progresses during telophase, under the influence of the contractile ring. The contractile ring squeezes the cytoplasm at the mid-circumference of the cell, creating what is called a **cleavage furrow**. The cell membrane is slowly pinched together at this point, resulting in the formation of two individual daughter cells. These events comprise the process known as **cytokinesis**.

### **Old Cells: The Effect of Cell Senescence**

It is a well-recognized fact that most somatic cell lines derived from mammals or birds possess a limited ability to replicate. That is to say that they will not continue to divide indefinitely, even when meticulously supported in vitro. This is in contrast to normal germ cells, and to cell lines developed from cancer cells, or other cells that have undergone a genetic mutation that makes them **immortal**.

Cells, which are derived from a normal fetus or young organism, will progress through more cell divisions than those taken from an adult organism. Additionally, cells derived from an adult will complete more cell cycles than those harvested from an elderly individual. Once these cells enter G<sub>0</sub>, they are unable to reenter the cell cycle. The dominant effect aging has on limiting the number of divisions a particular cell can accomplish has led to the term **cell senescence**.

The purpose of cell senescence and the exact mechanisms causing it remain a topic of intense research. Specialized, repetitive DNA sequences termed **telomeres** are found at the end of all chromosomes.<sup>37</sup> A unique enzyme called **telomerase** is responsible for directing the replication of telomeres during cell division.<sup>38</sup> This enzyme is not as precise as those which orchestrate the copying of the rest of the genome. This results in random differences in the number of telomeric repeats found at the end of each chromosomal DNA sequence. As a cell ages, there is a tendency for the telomeric sequences to be shortened. Therefore loss of telomerase is associated with cell senescence. This may be due to a relative deficiency of telomerase in somatic cells.

### **Clinical Applications: Cancer Chemotherapy**

An understanding of the cell cycle and its controlling mechanisms is important from both a scientific standpoint and from a more practical, clinical perspective. Many scenarios in clinical practice involve the processes of the cell cycle, from the healing of a



gastrointestinal anastomosis to the closure of a venous stasis ulcer. Direct use of the principles of the cell cycle in therapy is demonstrated no better than in the delivery of phase-specific cancer chemotherapy.

Many chemotherapeutic agents in clinical use act during a specific phase of the cell cycle, or direct a preponderance of their effects against an element of the cell cycle or its control system. In the sections below, we briefly discuss some chemotherapeutic agents and their phase-specific effects.<sup>39</sup>

### G1 and S Phase

The most important events in these phases of the cell cycle include passage of the G1 checkpoint, which will usually commit a cell to complete the cell cycle, and the synthesis of DNA. New strategies aimed at cyclin dependent kinases that are required for the transition of the G1 to S phase are being investigated. Chemotherapeutic agents which cause **DNA alkylation**, like dacarbazine and mechlorethamine, and those which interfere with **DNA synthesis**, like methotrexate and 5-fluorouracil, are active during these phases.

### M-Phase

The cell divides during M phase. In the previous sections, we described the numerous alterations in cytoskeletal arrangements, and in the configuration and alignment of chromosomes, that occur during M phase. Drugs, such as, taxol, vincristine, and vinblastine bind **tubulin**, a cytoskeletal protein important in the construction of microtubules. Agents like etoposide and doxorubicin inhibit **topoisomerase II**, an enzyme responsible for the "unwinding" of DNA. Recently two agents olomoucine and roscovitine have been shown to inhibit Cdk activity, which as explained earlier has important implications in the passage of a cell through the G2/S and G2/S checkpoints.<sup>40</sup>

Combination chemotherapy has been employed in many malignancies, utilizing arrays of drugs designed to inflict damage during both phases. For example, acute lymphocytic leukemia has been treated with combinations of prednisone, L-asparaginase, and cytosine arabinoside(**G1/S**), along with vincristine, daunorubicin, and etoposide (**M phase**).

Recently, new advances in the area of transcription and the roles of transcription termination and antitermination have been investigated. One of the areas studied has been the RNA polymerase subunit and how it is disrupted by DNA termination sequences. This area may offer an understanding on how tumor cells are able to bypass these termination sequences and continue to make the gene product.<sup>41</sup>

### Summary

The cell cycle and the cell cycle control system are the engines that drive life. They allow for the processes of cell renewal and the growth of organisms, under controlled conditions. The control system is essential for the monitoring of normal cell growth and replication of genetic material and to ensure that normal, functional daughter cells are produced at completion of each cell cycle.

Although certain clinical applications exist which take advantage of the events of the cell cycle, our understanding of its mechanisms and how to manipulate them is infantile. The next decades will continue to see the effort of many researchers focused upon unlocking the mysteries of the cell cycle and the cell cycle control system.

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